# EXHIBIT A

**PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| In re Application of:<br>Philip Ashton-Rickardt | Group Art Unit: 1642 |
| Serial No.: 10/782,401 | Examiner: Brandon J. Fetterolf |
| Filed: February 19, 2004 | Atty. Dkt. No.: ARCD:390US |
| For: METHODS AND COMPOSITIONS FOR THE INHIBITION OF CATHEPSINS | |

CERTIFICATE OF ELECTRONIC SUBMISSION

DATE OF FILING _____

## DECLARATION OF RAYMOND M. WELSH, PH. D.

I, Raymond M. Welsh, Ph.D., do declare that:

1. I am a United States citizen residing at 76 South Quinsigamond Ave., Unit 4, Shrewsbury, Massachusetts, 01545.

2. I currently hold the position of Professor, Department of Pathology, University of Massachusetts Medical Center (Worcester, MA). A copy of my National Institutes of Health (NIH) Biographical Sketch is attached as Appendix A, and a copy of my curriculum vitae is attached as Appendix B. Appendix B includes a numbered list of my publications.

3. I am a skilled virologist and immunologist and have expertise in the pathogenesis of viral infections. I understand the immunology of LCMV, as evidenced by the following:

   - I have worked with LCMV for over thirty-five years (since 1969), and I have collaborated and published with Nobel Lauriat Rolf Zinkernagel, whose work

pertaining to LCMV is quoted and presented below. I have written many reviews on the topic of LCMV, including the chapter on LCMV in the Encyclopedia of Virology.

- I am Editor for viral immunology and pathogenesis articles for the Journal of Virology, and am in charge of the review of many of the papers on the immune response to viruses, such as LCMV.
- I study and have NIH grants on the topic of apoptosis of T cells and on T cell memory. In addition, in 2004 I received a 10 year MERIT award from the National Institutes of Allergy and Infectious disease to continue my NIH-supported studies on T cell apoptosis during viral infections.
- I published some of the first work on LCMV-induced T cells having enzyme-containing granules (reference #102 in Appendix B) and in documenting apoptosis as a regulator of T cell responses during LCMV infection (*e.g.*, references #121 and 136 in Appendix B). I also published the first comprehensive review on apoptosis and viral infections (ref 130).
- In addition, I have published a paper in the journal, *Immunity*, on T cell apoptosis in the LCMV system and in analyzing granzyme mRNA levels within these T cells (reference # 189 in Appendix B). I have also recently published two papers in the Journal of Immunology, one (ref #203) linking resistance to apoptosis to the generation and recall of memory T cells and the other (ref #217) documenting an apoptosis-dependent loss in T cell memory under conditions of viral infection. Further, I have written a recent invited review in Current Opinion in Immunology (ref #205) entitled "Apoptosis and loss of virus-specific CD8 T cell memory."

4. I have reviewed U.S. Patent Application Serial Number 10/782,401 (hereinafter the "Application") and the pending claims. That application has a priority date of February 19, 2003.

5. I understand that the Examiner has rejected the pending claims of the Application because the Examiner believes that the specification does not reasonably provide enablement for an *in vivo* method of modulating cell death in a human by treating the human with an Spi2A polypeptide. In particular, the Examiner has argued that the specification is silent on any correlation between *in vitro* testing and *in vivo* use. The Examiner also argues that a considerable amount of *in vitro* testing is required, with no expectation of success being present, before Spi2A can be considered useful for treating a subject with a disease.

6. A skilled immunologist with an ordinary understanding of immunology would have recognized, at the of the priority date of the Application, that LCMV infection in mice is a model for many human diseases associated with T cell-mediated immunopathology. Examples of such diseases include, but are not limited to, diseases associated with increased lysosomal permeability, diseases associated with autophagic cell death, diseases associated with cell death mediated by TNF-α, diseases associated with reactive oxygen species, and diseases associated with necrosis. Specific examples of such diseases are set forth in the pending claims, and include infectious diseases, septic shock, hepatic failure, inflammatory diseases, liver disease, vascular disease, cardiovascular disease, cancer, bone disease, emphysema, neurodegenerative disease, viral infections, AIDS, immune disorders such as autoimmune disease, muscular dystrophy, and arthritis.

7. My positions with respect to the accepted nature of the LCMV mouse model is supported by literature that would be familiar to one having an ordinary understanding of immunology and virology. For example, the following references provide facts in support of the correlation between LCMV infection and different diseases:

**Zinkernagel, Vaccine 20:1913-1917, 2002 (Exhibit 1)**: This reference indicates that infection of adult mice with LCMV causes a more or less severe T cell mediated immunopathology, and that the organ that is primarily infected by the virus then determines the immunopathological disease. Examples of such diseases include choriomeningitis, hepatitis, graft-versus-host disease, and AIDS. See, *e.g.*, page 1914.

**Klenerman and Zinkernagel, Immunological Reviews 159:5- 16, 1997 (Exhibit 2):**

- Regarding the state of knowledge pertaining to LCMV infection in the mouse:

"[T]his infectious model has been established for over 60 years. The *in vivo* roles of specific immune subsets in the clearance of virus and the induction of disease are well understood." Page 5.

- Diseases of the meninges, liver, and lymph nodes: "In LCMV, the mediators of disease are CTL – whether this is in the meninges, the liver or the lymph node (85-94)." Page 13.

- Immunodeficiency states: "Destruction of the APC and lymphoid architecture by CTL-mediated lysis can lead to functional immunodeficiency during acute disease (95, 96)." Page 13.

- HIV: Regarding the rationale for comparing the LCMV mouse model to HIV:

"The reason for embarking on such a comparison is that the dominant immune response to both viruses is the cytotoxic T lymphocyte (CTL), and particular features of this immune response have striking parallels in the two infections. Since the CTL response to LCMV has been studied in immense detail, and its role *in vivo* has been accurately determined both qualitatively and quantitatively, it

provides an excellent reference point from which to view the role of the same cellular response in HIV." Page 6.

**Borrow *et al.*, J. Virology, 69:1059-1070, 1995 (Exhibit 3)**: This reference addresses the virus-induced immunosuppression induced by LCMV, the role of virus tropism in determining pathogenicity, and the role of dendritic cells. Similarities of LCMV to HIV are discussed.

- Abstract: "Our findings illustrate the key role that virus tropism may play in determining pathogenicity and, further, document a mechanism for virus-induced immunosuppression which may contribute to the clinically important immune suppression associated with many virus infections, including human immunodeficiency virus type I."

- Pages 1068-69: "Can our finding that virus infection of dendritic cells is a critical step in the production of immune suppression by LCMV clone 13 be generalized to other virus infections? It is of interest that all viruses known to be able to persist *in vivo* have been shown to infect cells of the immune system. . . In view of the central location of [dendritic cells] within the immune system and their unique, critical functions in the initiation of immune responses, it is likely that virus infection of dendritic cells and subsequent impairment of their functions will prove to be an underlying factor in many examples of generalized immune suppression associated with virus infection."

**Ciurea *et al.*, Proc. Natl. Acad. Sci. USA, 96:11964-11969, 1999 (Exhibit 4)**: This paper discusses the persistence of LCMV at very low levels in the immune mouse, and compares the results to infections with viruses and bacteria:

- Regarding similarities of LCMV to HIV and other infectious agents (abstract):

  "The finding that LCMV-WE persists in the face of apparently intact immune responses resembles the situation in some viral (hepatitis B and C, HIV) and bacterial (tuberculosis, leprosy) infections in humans; the results are relevant to the understanding not only of other murine and human persistent viral infections but also of protective immunological memory by 'infection immunity.'"

**Odermatt *et al.*, Proc. Natl. Acad. Sci. USA, 88:8252-8256, 1991 (Exhibit 5)**: This paper shows that LCMV-induced acquired immune suppression in mice is caused by $CD8^+$-T-cell-dependent elimination of macrophages/antigen-presenting cells (abstract).

- A comparison to HIV is discussed on page 8254-8255:

> "A possible $CD8^+$-T-cell-dependent pathogenesis of AIDS has been proposed to explain reduction of infected or HIV-antigen-binding $CD4^+$ T cells. It is conceivable that in analogy to the immunopathology observed during a LCMV infection, virus-specific cytotoxic T cells (and probably not the virus itself) may be responsible for both numerical and functional reduction of macrophages and antigen-presenting cells and thus cause destruction of follicular structures in HIV infections. Detailed histopathological studies may be taken to support the hypothesis of $CD8^+$-T-cell-dependent immunopathology may significantly contribute to the pathogenesis of AIDS; lymph node histopathology in patients with AIDS-related complex is often strikingly similar to that of mice suffering from LCMV-induced immunosuppression shown here."

**Khanolkar *et al.*, Immunol Res. 2002; 26(1-3):309-21 (Exhibit 6)**

- Regarding viral infections, this paper sets forth in the abstract that:

> "Lymphocytic choriomeningitis virus (LCMV) infection of mice has proven to be one of the most informative experimental systems for examining antiviral T cell responses." Abstract.

- Regarding MHC disease, on page 310, it is noted that:

> "Although LCMV is a relatively simple virus, encoding only four gene products, it has proven to be one of the best experimental systems for analyzing cellular immune responses. Studies on the immune system to this virus have been extremely informative, providing a foundation for our understanding of many fundamental immunologic concepts including major histocompatibility complex (MHC) restriction, tolerance, cytotoxic T lymphocyte activity, and immunologic memory."

- Regarding tumors and other viral infections, on page 319, it is noted that:

> "Overall, these findings illustrate that observations in the LCMV system provide a useful platform for the comparative analysis of cellular immunity induced by other viral infections as well as tumors."

**Hotchin J., Cold Spring Harbor Symp. Quant Biol. 27:479-99, 1962 (Exhibit 7)** – Regarding LCMV infection as a model for human autoimmune disease:

> "The proposed theory of acute and chronic virus induced autoimmune disease has profound implications for human chronic disease etiology in the field of the autoimmune diseases. The murine LCM virus system as a model offers experimental evidence that both early and later life virus infection can cause so-called idiopathic diseases." Page 497.

**Zinkernagel *et al.*, J Exp Med 1986; 164:1075-1092 (Exhibit 8)** – This paper shows that LCMV can cause a "virus-triggered but T cell-mediated liver disease [that] resembles the pathophysiology of acute hepatitis B virus infections in man(11-13), and may therefore serve as an animal model of its immunological pathogenesis." Abstract. It is further noted that "LCMV-WE-induced hepatitis in mice is an immunopathologically mediated disease caused by T cell-mediated destruction of infected liver cells" which "parallels many aspects of acute viral hepatitis in humans, which is caused by hepatitis B virus." Page 1090.

**Kagi *et al.*, J. Exp. Med. 1996; May 1; 183(5):2143-52 (Exhibit 9)** – Regarding LCMV infection in mice as a model for diabetes, this paper shows that in transgenic mice expressing glycoprotein of LCMV-GP in the β cells of the pancreas, "LCMV infection leads to a potent LCMV-glycoprotein-specific T cell response resulting in rapid development of diabetes." Abstract. It is further noted that "T cells play an important role in the destruction of beta cells leading to autoimmune type I diabetes." Abstract. The authors conclude that "we have shown here for a model system of autoimmune diabetes that insulitis is not dependent upon tissue damage by perforin-dependent

cytotoxicity, that perforin, which is expressed mainly by CD8+ T cells in islet infiltrates, plays an important role in the destruction of islet β cells and that other mechanisms or soluble factors produced by T cells or macrophages fail to cause elimination of β cells with similar efficiency." Page 2150.

**Jaeckel *et al.*, Annals of the NY Acad Sci 958:7-25, 2002(Exhibit 10)** – This paper reviews transgenic mouse models of diabetes where LCMV induces immunopathology (infiltration by lymphocytes, activation of antigen presenting cells, and expression of inflammatory cytokines) in transgenic strains of mice that express a viral "self-antigen." See abstract, and pages 17-19.

**Ohashi *et al.*, J. Immunol. 1993 Jun 1; 150(11):5185-94 (Exhibit 11)** – This reference teaches that "a transgenic mouse model has been established in which the lymphocytic choriomeningitis virus (LCMV) glycoprotein (gp) is expressed in the beta-islet cells of the pancreas (rat insulin promoter (RIP)-gp)." Abstract. "These mice (H-2b) do not spontaneously develop diabetes; however, infection with the LCMV strain WE rapidly induces hyperglycemia." Abstract.

**Evans *et al.*, J. Exp. Med. 1996 Dec 1; 184(6):2371-84 (Exhibit 12)** –This study describes "a transgenic mouse model in which peripheral infection by a virus [LCMV] that shares immune epitopes with a protein expressed in oligodendrocytes led to CNS autoimmune disease." Page 2379; see also abstract and page 2371. It is noted that "[t]he enhanced disease observed after a second LCMV infection shares several characteristics

with the human CNS disease, MS [multiple sclerosis], including chronic perivascular and parenchymal infiltration of autoreactive T cells in the brain and spinal cord, lesions in myelin tracts associated with the uptake of myelin components ..." Page 2381.

**Stellrecht-Broomhall, Viral Immunol. 1991 Winter; 4(4):269-80 (Exhibit 13)** – This reference indicates that studies of LCMV infection in mice have shown it to be a 'Rosetta Stone' for [the] immunopathologist" and indicates that "the role of virus-specific cytotoxic T cells (19), as well as their H-2 restriction (41); the mechanisms of immune complex diseases (26); and the demonstration of the ability of viruses to distort cellular functions in the absence of cytotoxicity (25) have all emanated from the murine LCMV model." Page 269. This paper describes LCMV infection of C3H3B mice as a model for autoimmune hemolytic anemia. See page 278.

**Zinkernagel *et al.*, Nature 1985 Aug 29-Sep 4; 316(6031):814-7 (Exhibit 14)** – This reference sets forth that susceptibility to murine LCMV maps to class I MHC genes, and thus represents a model for MHC/disease associations. See, *e.g.*, abstract. Such MHC/disease associations are said to include "chronic diseases of autoimmune, immunopathological or cancerous nature." Page 817.

**Ludewig *et al.*, J. Exp. Med. 2000 Mar 6; 191(5):795-804 (Exhibit 15)** - Regarding LCMV infection in mice and cancer, this paper describes use of LCMV infection of RIP-GP mice implanted with MC-GP fibrosarcoma cells as a model for assessing efficacy of induction of anti-tumor immunity. See page 797.

**Oldstone MB, Prog Med Virol. 1975;19:84-119 (Exhibit 16)** – According to this review article, "[p]ersistent LCM viral infection of mice is the prototype experimental model for [the] study of V-Ab [viral – antiviral antibody] immune complex disease." Page 106. This reference describes similarity between the pathophysiology of LCMV and other diseases associated with immune complex deposition, including systemic lupus erythematosus and blood vessel disease such as vasculitis. Page 108-109.

**Nansen and Thomsen, J. Immunol. 2001, 166:982-988 (Exhibit 17)** – Regarding LCMV infection in mice as a model for septic shock, this references sets forth that "LPS is a major active agent in the pathogenesis of Gram-negative septic shock," and that "[a] shock-like state can be induced by a single injection of LPS into animals" and that "systemic infection of mice with the noncytopathogenic lymphocytic choriomeningitis virus (LCMV) sensitized mice to low amounts of LPS." Abstract and page 982.

8. Based on my review of these materials and my experience, a skilled virologist with an ordinary understanding of viral immunology would have understood, at the time the above-referenced application was filed, that LCMV infection in mice is an established model for a wide variety of diseases associated with immunopathology in humans.

9. My review of the specification of the application identified the following sections pertaining to LCMV infection and in vivo studies:

- Page 65, lines 21-28 – This section provides general information and guidance regarding generation of LCMV infection in mice.

- Example 3 (page 65, line 1 – page 94, line 4): This example provides information regarding the identification of Spi2A as a protective gene that facilitates the differentiation of memory T lymphocytes. LCMV was used to generate a stable pool of anti-LCMV memory CD8 cells based on techniques known to those of ordinary skill in the art. See page 83, lines 9-11. These cells were used in analyses to identify genes up-regulated in memory CD8 cells. Spi2A was found to be up-regulated to the greatest extent during the development of naïve to memory cells. Page 87, lines 9-11.
- Further, *in vivo* studies demonstrated that expression of Spi2A increased the percentage and absolute number of anti-LCMV CD8 cells in Spi2A mice in two independent experiments. See page 91, lines 16-18 and FIGS. 19, FIG. 20A, and Table 7. In addition, the effect of Spi2A was specific for antigen-specific CD8 cells. Page 92, lines 17-18 and FIG. 20B. Further, the specification shows that Spi2A had a dramatic effect on the recall response of CD8 cells to re-infection with LCMV in mice. Page 93, line 11 – page 94, line 4, FIG. 19, Table 7, FIG. 20.
- Example 6 (page 95, line 10 – page 96, line 6): This example provides guidance regarding *in vivo* studies to assess the prevention of tumor development using Spi2A polypeptides and Spi2A polypeptide equivalents.
- Example 7 (page 96, line 8 – page 97, line 8): The example provides guidance regarding clinical trials to evaluate the treatment of septic shock in human subjects using Spi2A polypeptides and Spi2A polypeptide equivalents.
- Example 9 (page 98, line 10 – page 99, line 15) – This example provides guidance regarding clinical trials pertaining to the treatment of cancer in human subjects using Spi2A polypeptides and Spi2A polypeptide equivalents.

- Example 11 (page 100, line 16 – page 101, line 26): This example provides general guidance regarding clinical trials pertaining to the use of Spi2A polypeptides and Spi2A polypeptide equivalents in the treatment of diseases.
- Example 12 (page 102, line 1 – page 103, line 15): This example provides general guidance regarding the establishment of clinical trials pertaining to the use of Spi2A polypeptides and Spi2A polypeptide equivalents in the treatment of cancer.
- Example 13 (page 103, line 16 – page 105, line 20): This example provides general guidance regarding establishing clinical trials pertaining to the use of Spi2A polypeptides and Spi2A polypeptide equivalents in the treatment of Alzheimer disease.
- Example 14 (page 105, line 22 – page 106, line 4): This example provides general guidance regarding the setting up of clinical trials pertaining to the use of Spi2A polypeptides and Spi2A polypeptide equivalents in treating liver disease.

10. Furthermore, following the filing of their patent application, the inventor's research group published a report **(Nature Immunology, 5(9):919-926, 2004; "Exhibit 18)** that provides information which supports the findings set forth in the application that Spi2A is a protective factor for memory T cell development. The paper presents results of the studies showing that the gene encoding Spi2A is upregulated in memory cell precursors. Exhibit 18, abstract and pages 920-922. It was also found that Spi2A upregulation protected LCMV-specific memory progenitors from programmed cell death. Exhibit 18, abstract, and pages 922-924. Thus, Spi2A promotes the survival of cytotoxic T lymphocytes, allowing them to differentiate into memory CD8 T cells.

11. The information set forth in the specification establishes that Spi2A plays a critical role as a protective factor that facilitates the differentiation of memory T-lymphocytes. The inventor's publication of Liu et al. (Exhibit 18) provides evidence supporting the benefit of Spi2A in inducing T-cell mediated immunity *in vivo*.

12. In view of the information set forth in the specification pertaining to the protective role of Spi2A in facilitating the differentiation of memory T-lymphocytes and the state of the art pertaining to LCMV, it is my belief that a person of ordinary skill in my field would understand that Spi2A will be of benefit in abrogating immunopathology associated with LCMV infection. Furthermore, a person of ordinary skill in my field, when presented with the present specification, would be able to practice the invention as claimed without an undue amount of experimentation. In view of the information set forth in the specification pertaining to the role of Spi2A in the induction of T-cell mediated immunity and the protective effect of LCMV-specific memory progenitors from programmed cell death, one of ordinary skill in the art would understand that Spi2A or Spi2A equivalents may be of benefit in the treatment of infectious disease, septic shock, hepatic failure, inflammatory diseases, liver disease, vascular disease, cardiovascular disease, cancer, bone disease, emphysema, neurodegenerative disease, viral infections, AIDS, immune disorders such as autoimmune disease, muscular dystrophy, and arthritis in humans, and that no undue experimentation would be required to practice the claimed invention.

13. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these

statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

January 2, 2007
Date

Raymond M. Welsh, Ph.D.